1. Tube Dilution Method:

- Tube dilution method is the most commonly used method for determination of **minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC).** In this method, a series of tubes is set up, each containing the same quantity of **a standard growth liquid medium** and also a gradually increasing **concentration of the antimicrobial chemical agent** (e.g., antibiotic) to be tested.

- The tubes are then **inoculated** with the same quantity of cell suspension of the test microorganism and **incubated** till growth has appeared (usually 16 to 20 hours).
- The first tube in the series where **there is complete absence of growth of** the test microorganism denotes (mark) **the minimal inhibitory concentration (MIC)** of the chemical agent.

- **The minimal lethal concentration (MLC)** is determined by taking small quantities (usually 0.05 ml) from the tubes **showing no growth** and **sub culturing** it into fresh medium lacking the chemical agent.

- The lowest concentration of the agent from which **the microorganism does not recover** and **grow** when transferred to fresh medium is the minimal lethal concentration (MLC). The tube dilution method for determination of MIC and MLC is diagrammatically represented in Fig. 20.1.

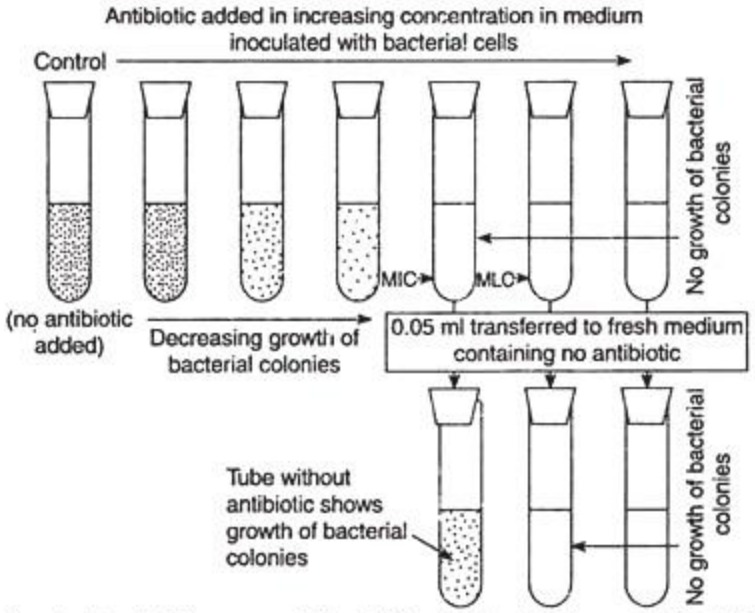

FIG. 20.1. Determination of minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) by the tube dilution method.

2. Agar Plate Method:

- Agar plate method (Fig. 20.2) is very similar to tube dilution method.
- In this method, Petri plates containing a standard growth agar medium are taken at the place of lubes containing liquid medium.
- Antimicrobial chemical agents of various concentrations are inoculated on the agar surface of plates already inoculated with the same quantity of the test microorganism.
- These plates are incubated and then examined for growth.
- The first plate in the series where there **is complete absence of growth** of the test microorganism denotes the minimal inhibitory concentration (MIC) of the chemical agent.

- Also, the chemical agent to be tested may be applied in the center of the Petri dish and **zone of inhibition** can be observed. **Zone of inhibition develops if the chemical agent is active.**

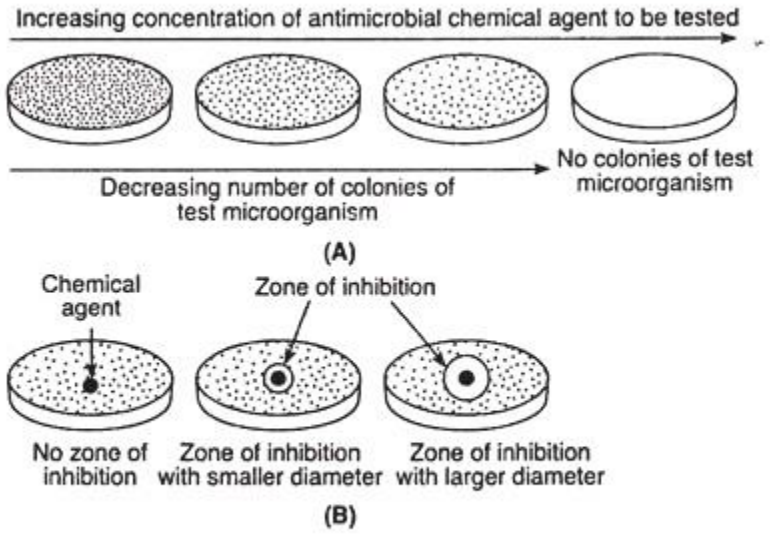

FIG. 20.2. Evaluation of antimicrobial chemical agent in laboratory. **(A)** Chemical agent evenly distributed on agar surface and **(B)** chemical agent applied to centre of inoculated medium in Petri dish.

Phenol-Coefficient Method:

Suitable for disinfectant miscible with water

Strain use is **Salmonella typhi or Staphylococcus aureus**

- To determine the phenol coefficient, different dilutions of phenol and test disinfectants (5 ml per tube) are added separately to tubes containing 0.5 ml of 24 hour **old broth culture** (test culture) of Staphylococcus aureus or Salmonella typhi.

- All these tubes are then placed in a 20°C water both.
- At intervals of 5, 10 and 15 minutes, an aliquot (portion) from each tube is transferred to a nutrient broth medium with a loop transfer needle for sub-culturing.

- The inoculated subculture tubes are incubated for 2 days and subsequently examined for visible growth.
- The highest dilution of the disinfectant and the highest dilution of phenol killing the test organism (Staph, aureus or Sal. typhi) in 10 minutes but hot in 5 minutes are recorded.
- The number obtained by this division is the phenol coefficient of the substances tested.